IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Grotendorst, et al.
Serial No.: 08/386,680
Filed : 2/10/95
Title : **Connective Tissue Growth Factor**

Art Unit: 1812
Examiner:L. Spector

Commissioner of Patents and Trademarks
Washington, DC 20231

DECLARATION UNDER 37 C.F.R. §1.131

Dear Sir:

I, Gary R. Grotendorst, Ph.D. declare and state that:

1. I am a co-inventor of the subject matter described and claimed in the United States Patent Application Serial No. 08/386,680, filed February 10, 1995 entitled, "Connective Tissue Growth Factor".

2. I am familiar with the prosecution history of Patent Application Serial No.08/386,680.

3. I understand that the Examiner has rejected claims 5-12 under 35 U.S.C. §102(a) as anticipated by Ryseck, et al. (Cell Growth & Differentiation, 2:225).

4. The clone and sequence of CTGF were obtained in my laboratory in the United States and submitted to GenBank on July 17, 1990, prior to the May 1991 publication date of Rysek. The Office Action states that a comparison of the amino acid sequence of fisp-12 and CTGF reveals only 13 discrepancies in the region between 86 to 392. The Office Action states that there is greater divergence in the region preceeding residue 86. The Office Action states that Ryseck identifies this region as a signal sequence which would not affect protein activity.

5. I disagree with the conclusions stated in the Office Action. It is well known in the art that a typical signal sequence is about 15-25 amino acids in length. In fact, on page 227 of Ryseck, line 5, the authors state that the signal sequence of fisp-12 is only 21 amino acids (also see FIGURE 3). The cleavage site for the signal sequence is between residues 25 and 26 (page 226, column 2, second paragraph). Therefore, the sequence divergence found in amino acids 26-86 is significant and therefore the fisp-12 protein described by Ryseck is distinguishable from CTGF of the present invention.

6. Further, prior to the May, 1991 date of the Ryseck reference, I had immunoaffinity purified CTGF and shown that it had mitogenic activity in a DNA synthesis assay using NRK fibroblasts. EXHIBIT A shows laboratory notebook pages from my lab for experiments which were performed prior to the date of the Ryseck reference showing that immunoaffinity purified CTGF has mitogenic activity.

7. Briefly, serum free (S/F) media from cultured HUVE cells was affinity purified on a column of Affi-Gel-10 conjugated with anti-PDGF IgG by methods described in Matsuoka, *et al.* (cited in this Office Action). Affinity purified material was analyzed in a Western blot and in a mitogenic assay using NRK cells as described by Matsuoka, *et al.* The data shown in EXHIBIT A, page 2 (table of cpm/sample) indicate that the affinity purified material (see for example samples 7-13) had mitogenic activity comparable to purified PDGF (samples 14-17).

Western blot analysis of the affinity purified mitogenic fractions revealed a protein with mitogenic activity that migrated at about 36 kDa. This protein fraction was identified as CTGF.

8. The identification of PDGF-like activity in HUVE cell conditioned media prompted the cloning and the isolation of a full length CTGF clone from a HUVE cell library (see Examples of the present patent application). The clone, designated DB60, was isolated from a HUVE cell cDNA library in λgt11 screened with anti-PDGF antibody. Anti-PDGF antibody binding to the fusion protein produced by the clone DB60 was

completely blocked by the affinity purified proteins. A Northern blot analysis using RNA from HUVE cells indicated that the clone hybridized with a mRNA of about 2.4 kb, which is a message of sufficient size to produce a protein in the 38 kD molecular weight range as seen on the immunoblots of the affinity purified proteins.

The clone encoding the entire open reading frame of the CTGF protein as identified by these biological characteristics, was isolated prior to the May, 1991 date of the Ryseck reference.

9. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date Jan 4, 1996

Gary R. Grotendorst, Ph.D.